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GEOGRAPHIC VARIATION IN ALKALOID PRODUCTION IN *Conium maculatum* POPULATIONS EXPERIENCING DIFFERENTIAL HERBIVORY BY *Agonopterix alstroemeriana*

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Abstract—*Conium maculatum*, a Eurasian weed naturalized in North America, contains high concentrations of piperidine alkaloids that act as chemical defenses against herbivores. *C. maculatum* was largely free from herbivory in the United States, until approximately 30 yr ago, when it was reassociated via accidental introduction with a monophagous European herbivore, the oecophorid caterpillar *Agonopterix alstroemeriana*. At present, *A. alstroemeriana* is found in a continuum of reassociation time and intensities with *C. maculatum* across the continent; in the Pacific Northwest, *A. alstroemeriana* can cause severe damage, resulting in some cases in complete defoliation. Studies in biological control and invasion biology have yet to determine whether plants reassociated with a significant herbivore from the area of indigeneity increase their chemical defense investment in areas of introduction. In this study, we compared three locations in the United States (New York, Washington, and Illinois) where *C. maculatum* experiences different levels of herbivory by *A. alstroemeriana* to determine the association between the intensity of the interaction, as measured by damage, and chemical defense production. Total alkaloid production in *C. maculatum* was positively correlated with *A. alstroemeriana* herbivory levels: plants from New York and Washington, with higher herbivory levels, invested two and four times more N to alkaloid synthesis than did plants from Illinois. Individual plants with lower concentrations of alkaloids from a single location in Illinois

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experienced more damage by *A. alstroemeriana*, indicative of a preference on the part of the insect for plants with less chemical defense. These results suggest that *A. alstroemeriana* may act either as a selective agent or inducing agent for *C. maculatum* and increase its toxicity in its introduced range.

Key Words V Insect¥plant interactions, *Conium maculatum*, *Agonopterix alstroemeriana*, chemical defenses, alkaloids, γ-coniceine, coniine, conhydrinone, evolution, herbivory.

INTRODUCTION

Flowering plants and the insects that eat them collectively constitute almost half of all terrestrial species (Berenbaum, 1995). Interactions between plants and herbivorous insects are of central importance in determining community structure in a wide range of terrestrial systems. Thus, perturbations that affect interactions can have profound impacts within terrestrial systems. Among the global perturbations of increasing concern is the introduction and incorporation of nonnative species into communities within which they have not evolved. Once established, invasive plants can cause direct economic damage by reducing crop yields and livestock growth, and indirect damage by altering community composition via displacement of native species (Vitousek et al., 1996; Pimentel et al., 2000). Invasive species can influence the evolution of native species via niche displacement, competitive exclusion, introgression, hybridization, and even extinction (Mooney and Cleland, 2001).

Because the rate at which invasive species enter and become established in the United States has been increasing (Mooney and Cleland, 2001), there is considerable interest within the scientific community in understanding the dynamics of invasion. Characterizing "invasibility,^ however, has proven difficult (Crawley, 1987; Rejmanek and Richardson, 1996; Reichard and Hamilton, 1997). Transport of a species out of its native habitat generally results in a reduction in herbivory by coevolved specialist insects. Indeed, the idea that plant populations in areas of indigeneity are regulated by herbivores underlies the practice of classical biological control of weeds (Huffaker, 1959; Willis et al., 2000). The fact that plants tend to grow taller and produce more seeds in areas of introduction than in areas of indigeneity has been attributed in part to release from herbivorous natural enemies (Crawley, 1987). Blossey and Nötzold (1995), in proposing what they call the evolution of increased competitive ability (EICA) hypothesis based on optimal defense theory (Zangerl and Bazzaz, 1992), argue that invasiveness results from changes in biomass allocation patterns; in areas of introduction, where herbivores are absent, genotypes with reduced resource allocation to herbivore defense and increased resource allocation to competitive abilities are favored. Although

preliminary tests of this hypothesis with purple loosestrife (*Lythrum salicaria*) were suggestive (Blossey and Nötzold, 1995) and have been confirmed in other systems (Siemann and Rogers, 2001; Blair and Wolfe, 2004), additional tests failed to document intraspecific variation in herbivore resistance according to plant origin (Willis et al., 1999; Stastny et al., 2005), and a broader survey suggests that differences in sizes of plants in indigenous vs. nonindigenous habitats may represent plastic phenotypic variation rather than evolutionary change (Willis et al., 2000). More recently, a comprehensive comparison of size, fecundity, and leaf areas of nonindigenous and indigenous populations of *Hypericum perforatum* provided compelling evidence of the capacity for rapid contemporary evolution of these traits in invasive species (Maron et al., 2004a).

In general, little quantitative and ecologically relevant information is available on phytochemical changes in plants that occur after introduction into a nonindigenous area and release from interactions with longtime insect associates (Daehler and Strong, 1997; Willis et al., 1999; Siemann and Rogers, 2003; Maron et al., 2004b). Perhaps equally important, little information is available on phytochemical changes that ensue when coevolved herbivore associates resume interacting with a host plant in a nonindigenous area. This scenario is of no small consequence in that classical biological control involves reconstructing such plantYherbivore associations in the area of introduction. The possibility exists that, in the area of introduction, a newly resumed interaction will differ dramatically in its dynamics from such interactions in the area of indigeneity, given the differences in the structure of the surrounding community. Understanding the selective impact of reassociated herbivores on the chemistry of their host plants in areas of introduction is of interest not only in the context of understanding the basic dynamics of plantYinsect interactions, but also in predicting potential trajectories of classical weed biological control programs.

A system in which the chemical consequences of reassociation with a coevolved herbivore may be thoroughly examined involves the interaction between *Conium maculatum* (L.) (Apiaceae) (poison hemlock), a Eurasian weed, and its monophagous associate *Agonopterix alstroemeriana* (Clerck) (Lepidoptera: Oecophoridae), a leaf-rolling European caterpillar known only to feed on *C. maculatum*. *C. maculatum* is an herbaceous Eurasian biennial that is extensively naturalized in temperate North America, as well as in other parts of the world, including Australia, New Zealand, and South America (Parsons, 1976; Holm et al., 1979). The weed is generally regarded as noxious; all aerial parts are poisonous to livestock and to humans (Sperry et al., 1964; Widner, 1984; Markham, 1985; Hannam, 1985; Jessup et al., 1986; Panter et al., 1988; Panter and Keeler, 1989). The toxicity of *C. maculatum* to vertebrates is attributable primarily to its production of relatively high concentrations of coniine

and related piperidine alkaloids, including methylconiine, coniceine, and conhydrine (Fairbairn, 1971). Its tendency to invade fields of alfalfa and other forage crops has led to livestock death through contamination of green-chopped hay (Kubik et al., 1980; Panter et al., 1988). *C. maculatum* is frequently a target of eradication programs in populated areas because of its toxicity, as well as to its rank odor and profuse growth.

Relative to other introduced weed species, *C. maculatum* is attacked by few insect herbivores. In an extensive survey of poison hemlock in southern California, Goeden and Ricker (1982) reported "amazingly few insect species or individuals thereof. A clear majority, 16 (70%) of the 20 [*sic*] phytophagous insect species found on this weed were rare and were only encountered as a few individuals at one or two sites.^ Of the relatively few native insect species that have colonized the plant extensively throughout its range, the majority are species that feed generally on native and introduced plants in the Apiaceae; these species include *Papilio zelicaon* Lucas (Goeden and Ricker, 1982), *P. polyxenes asterius* Stoll (Feeny et al., 1985; personal observations) (Lepidoptera: Papilionidae), and *Euleia fratria* (Loew) (Diptera: Tephritidae) (Berenbaum, 1981; personal observations). The most abundant insect associate of the plant in California until recently has been an aphid, *Hyadaphis foeniculi* (Passerini), accidentally introduced from Europe, where it also feeds on poison hemlock (Goeden and Ricker, 1982).

A. alstroemeriana, a leaf-rolling oecophorid caterpillar monophagous on *C. maculatum* throughout its native range in Europe, was first reported on *C. maculatum* populations in the United States in Tompkins County, NY, in 1973 (Berenbaum and Passoa, 1983). *A. alstroemeriana* was subsequently reported in 1983 in northern California, Oregon, and Utah and by 1987 was established in mesic areas of Washington, Idaho, and Colorado (Powell and Passoa, 1991), where it remains reliably "collectible in large numbers^ (Anonymous, 1995). An adult *A. alstroemeriana* collected in 1990 near Columbus, OH, marked the first appearance of this species in the Midwest (Powell and Passoa, 1991). In 1993, the existence of established populations of *A. alstroemeriana* in central Illinois was confirmed (Berenbaum and Harrison, 1994); McKenna et al. (2001) reported substantial populations of this insect at several sites throughout Champaign County, IL.

Thus, throughout its range in North America, populations of *C. maculatum* exist with varying histories of reassociation with a specialist herbivore from its area of indigeneity. This continuum of association allows us to test whether a specialist insect (and potential biological control agent) may act as a selective agent on the defense chemistry of its host plant. In this study, we set out to determine whether chemical defense production by *C. maculatum* changes in response to reassociation with a principal herbivore from its area of indigeneity.

METHODS AND MATERIALS

Sampling. Study sites were located in Champaign County, IL, USA (40.109°N, 88.203°W), Tompkins County, NY, USA (42.443°N, 76.503°W), and Whitman County, WA, USA (46.733°N, 117.161°W). These locations were selected because they represent a continuum in time of association with and intensity of herbivory by *A. alstroemeriana* on *C. maculatum*, with longer association and higher levels of herbivory in Washington and New York and shorter association and lower levels of herbivory in Illinois. In June 2003, we selected four sites located more than 2 km apart within each region. *A. alstroemeriana* larvae can be found on *C. maculatum* for a short period of time, between 30 and 45 days, with some differences in life cycle timing among the regions studied. Thus, *A. alstroemeriana* larvae can be found from late April to early June in Illinois (Berenbaum and Harrison, 1994), from early May to mid-June in New York (Berenbaum and Passoa, 1983), and from early June to mid-July in Washington (Piper, personal observation). *C. maculatum* sampling was conducted in early June in Illinois and New York and in late June in Washington to coincide with the presence of *A. alstroemeriana* larvae. Plants are in approximately the same developmental stage across regions when *A. alstroemeriana* is abundant (early to mid-flowering). At each site within a region, we estimated *A. alstroemeriana* damage levels. Five levels of intensity were identified: level 0, *A. alstroemeriana* leaf rolls absent; level 1, leaf rolls present, but no visible leaf damage; level 2, minor defoliation, with up to one quarter of leaf area damaged (as estimated by eye); level 3, mild defoliation, between one quarter and one half of leaf area damaged; level 4, major defoliation, with more than one half of leaf area damaged; and level 5, complete defoliation, with most of leaf area damaged.

In each region, we randomly sampled between 12 and 20 *C. maculatum* plants per site. Two subsamples of green foliage per plant, one for alkaloid analysis and one for nitrogen and leaf water content analysis, were placed separately into Eppendorf tubes and frozen *in situ* on dry ice. Samples were shipped on dry ice to our UIUC laboratory and stored at −80°C until analyzed.

Plant Extraction and Chemical Analyses. Leaf material (ca. 200 mg FW) was ground using a ball mill and extracted on a shaker for 1 hr with 1.5 ml of acidified methanol (70% MeOH, 30% 0.1 N HCl). After centrifuging, 1 ml of the resulting extract was concentrated to approximately 0.2 ml on a centrifugal evaporator (Jouan RC 10.10), extracted with hexane to remove nonpolar compounds, and placed back into the centrifugal evaporator to remove the residual hexane. The remaining solution was then basified with 10 M NaOH; these were extracted in 200 μl hexane with 0.01% hexadecane. Alkaloids were analyzed by flame ionization detection on a gas chromatograph equipped with capillary column (Alltech EC-1, 30 m, 0.23 mm) coupled with an autosampler

(HP 5890). Hexadecane was used as internal standard. The samples were run with the following temperature program: initial temperature 50°C, ramp 5°C min^{-1} up to 105°C, ramp 35°C min^{-1} up to 290°C, 5 min at 290°C. (±)-Coniine (Sigma) was used as a standard. Alkaloid concentrations were expressed as coniine equivalents on a dry weight basis and per milligram of nitrogen. Total alkaloid concentrations were calculated by adding the concentrations of each individual alkaloid.

Total nitrogen was measured to estimate the relative resource investment in defense compounds among the three regions studied. A subsample of fresh leaf material was weighed, oven-dried at 60°C overnight, and weighed again to obtain the FW/DW ratio. Samples (10 plants per site) were then ground and analyzed for total nitrogen in an elemental combustion analyzer (Costech Instruments ECS 4010).

Isolation and Identification of Alkaloids. Leaves of *C. maculatum* were collected during March 2004 at Phillips Tract Experimental Station located at 5 km of Urbana, IL, USA. Fresh leaves (200 g FW) were extracted in a blender with 70% methanol, 30% 0.1 N HCl and filtered through Whatman 1 filter paper. The residue was re-extracted two more times; fractions were bulked together (1 l in total) and filtered through reversed-phase C18 (Baker; 40 μm) previously rinsed with methanol to remove nonpolar compounds. The eluate containing the alkaloids was concentrated by rotary evaporator at low temperature (max 45°C) until the volume was reduced by half; the eluate was then partitioned with chloroform (3×'s) to further remove nonpolar compounds. In a separation funnel, the extract was basified with 10 M NaOH and liquidYliquid partitioning was conducted with chloroform (5×'s) to extract the alkaloids. The chloroform fractions were combined, mixed with 20% HCl in MeOH, and evaporated to obtain a mixture of alkaloids in hydrochloride form. Bulk alkaloids were resuspended in ethanol/0.1 N HCl (1:1), basified with 10 M NaOH, and extracted (3×'s) with a small volume of chloroform. Individual alkaloids were isolated using a 25 × 2.5 cm silica gel (Merck; 32Y63 μm) gravity column eluted with 150 ml of chloroform/ethanol/NH_3OH (70:30:1) (Leete and Olson, 1972) at ca. 1 ml min^{-1}. Fractions (2 ml each) were monitored by spotting 5 μl on a TLC silica gel plate (Baker; 250 μm) and sprayed with Dragendorff (Jungreis, 1985) or 0.2% ninhydrin reagents (Sigma). Although individual alkaloids did not completely separate, we could obtain fractions with more than 95% purity for the two major alkaloids to be identified by nuclear magnetic resonance (NMR) (RT 6.5 and RT 10) as explained below. The low concentration of alkaloid RT 12.0 did not allow us to obtain enough pure material for structure elucidation. Coniine identity (RT 5.6) (Figure 1) was established by comparison with authentic material (Sigma).

Liquid Chromatography-ElectroSpray Ionization-Mass Spectrometry (LC-ESI-MS) Analysis for Hemlock Alkaloids. Samples were run on a Finnigan-

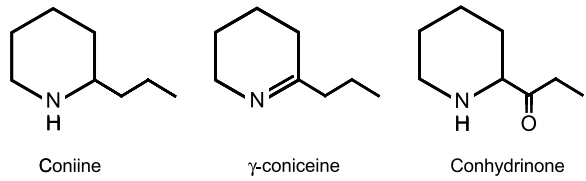


FIG. 1. Major piperidine alkaloids of *Conium maculatum* from Illinois, New York, and Washington.

Thermoquest LCQ LC-MS system (AS3000 autoinjector, P4000 HPLC pump, UV6000 PDA detector, LCQ mass spectrometer) (San Jose, CA) all running under the Xcaliber 1.2 software system. The MS was run with the ESI probe in the positive mode. The column was a 3 × 150 mm Inertsil reverse-phase C-18, ODS 3, 3-μm column (Metachem, Torrance, CA) with a Metaguard guard column. The source inlet temperature was set at 220°C, and the sheath gas rate was set at 90 arbitrary units. The MS was optimized for detection of hemlock alkaloids by using the autotune feature of the software while infusing a solution of coniine with the column effluent and tuning on an atomic mass unit of 128 $[M+H]^+$. The solvent systems were as follows: (A) water with 0.1 M ammonium acetate and 0.25% acetic acid; and (B) methanol with 0.1 M ammonium acetate and 0.25% acetic acid. The column was equilibrated with 2% B at a flow rate of 0.3 ml min^{-1}. After injection, the column was held at the initial conditions for 2 min, then developed with a linear gradient to 40% B over 23 min and then to 50% B over the next 10 min. The column effluent was monitored at 210 nm in the PDA detector. Mass data between 150 and 1000 AMUs was collected. Generally, the most significant sample ion generated under these conditions was $[M+1]^+$.

*Gas Chromatography*Y*Mass Spectrometry Analysis for Hemlock Alkaloids.* Gas chromatographyYmass spectrometry (GC-MS) was performed using a Hewlett-Packard (HP) 6890 GC system attached to an HP 5972A Mass Selective Detector. The column was a fused silica HP-5MS capillary (0.25-μm film thickness, 30 m × 0.25 mm ID). The GC operating parameters were as follows: splitless injection mode; temperature programmed from 50 to 315°C at 5°C min^{-1} with a 10-min initial temperature hold; He carrier gas flow rate at 1.1 ml min^{-1}, with the injector temperature set at 250°C. Spectra were compared to known standards or by computer with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer, 1989).

Nuclear Magnetic Resonance Analysis of Hemlock Alkaloids. ^1H, Correlation Spectroscopy (COSY), Distortion less Enhancement by Polarization Transfer (DEPT), Heteronuclear Single Quantum Coherence (HSQC),

and ^{13}C-NMR spectra were obtained with a Bruker (Billerica, MA, USA) Avance 500 spectrometer equipped with a 5-mm inverse broadband Z-gradient probe (^{13}C NMR, 125 MHz; ^{1}H, 500 MHz). Nuclear magnetic resonance spectra were recorded in methanol-d4, which served as the internal reference (^{13}C, 49.0 ppm; ^{1}H, 3.30 ppm). The data were analyzed using the Advanced Chemistry Development, Inc., SpecManager 1D Processor and the HNMR and CNMR Predictor software suite (Toronto, ON).

Structure Confirmation of γ-Coniceine (RT 6.5). Positive ion LC-ESI-MS showed the presence of one major compound with a large mass ion $[M+H]^+$ of *m*/*z* 126. Prominent diagnostic GC-mass spectral ions and their relative intensities are as follows: EI-MS [*m*/*z* (%)]: 125 (M^+, 13), 124 (10), 110 (32), 97 (100), 96 (43), 82 (10), 70 (19), 55 (10), 54 (10); ^{1}H-NMR δ (CD_3OD): 3.65 (2H, bs), 2.82 (2H, m), 2.62 (2H, m), 1.90 (2H, m), 1.86 (2H, m), 1.71 (2H, m), 1.02 (3H, t, J = 7.4 Hz); ^{13}C-NMR δ (CD_3OD): 45.9, 41.0, 30.6, 20.3, 20.3, 17.9, 7.4. The ^{1}H-NMR and ^{13}C-NMR spectra identify a compound with a composition of $C_8H_{15}N$ consistent with the structure of γ-coniceine (Asensio et al., 1993; Fukuda et al., 1991) (Figure 1).

Structure Confirmation of Conhydrinone (RT 10). Positive ion LC-ESI-MS showed the presence of one major peak with a large mass ion $[M+H]^+$ of *m*/*z* 142. Prominent diagnostic GC-mass spectral ions and their relative intensities are as follows: EI-MS [*m*/*z* (%)]: 141 (M^+, 2), 98 (100), 84 (4), 70 (8), 55 (65); ^{1}H-NMR δ (CD_3OD): 4.04 (1H, dd, J = 12.3 Hz, J = 3.3 Hz), 3.39 (1H, m), 2.99 (1H, m), 2.63 (1H, m), 2.38 (1H, m), 1.91 (2H, m), 1.67 (2H, m), 1.54 (1H, m), 1.09 (3H, t, J = 7.2 Hz); ^{13}C-NMR δ (CD_3OD): 207.4, 64.4, 44.9, 32.6, 27.1, 23.2, 23.0, 7.4. The ^{1}H-NMR showed signals for an isolated ethyl group adjacent to a carbonyl carbon. The downfield proton is consistent with a proton attached to the carbon in the ring that is both adjacent to the nitrogen and attached to the side chain bearing the ketone. All assignments for conhydrinone could be obtained from the COSY spectrum. The ^{13}C-NMR revealed the presence of a keto carbonyl at 207.4 ppm. All the assignments were obtained from DEPT and HSQC experiments. The spectra match that of a compound with a composition of $C_8H_{15}NO$ corresponding to conhydrinone (Leete and Olson, 1972) (Figure 1).

Information on an Unknown Alkaloid (RT 12). Positive ion LC-ESI-MS showed the presence of one major compound with a large mass ion $[M+H]^+$ of *m*/*z* 126 and several minor contaminating TIC peaks. The retention time of this compound in the GC is significantly longer than of γ-coniceine; on this basis, we conclude that the compound has a different chemical structure. Prominent diagnostic GC-mass spectral ions and their relative intensities are as follows: EI-MS [*m*/*z* (%)]: 125 (M^+, 1), 124 (6), 110 (18), 97 (100), 96 (31), 82 (9), 69 (7), 55 (15). Examination of purified fractions containing this peak by NMR and IR yielded conflicting data as to the exact chemical structure of this compound.

At this time, we can say it appears to be a coniceine isomer with the exact location of the double bond undetermined.

Herbivory and Alkaloids. To determine whether *C. maculatum* chemistry is associated with differences in *A. alstroemeriana* abundance, we randomly selected 29 plants from one site in Champaign County, IL ("Yard waste site^) in June 2003. For each plant, we counted the number of leaf rolls; two leaf samples per plant were then taken and alkaloids were analyzed as described. The number of leaf rolls was used as an estimate of herbivory intensity assuming a proportional relationship between the number of larvae and the number of leaf rolls made during larval development.

Statistical Analyses. All statistical analyses were performed using Statistica 6.0 (Statsoft, Tulsa, OK, USA). A one-way analysis of variance with "site^ nested in "region^ (Illinois, New York, and Washington) was performed to examine differences among populations in total alkaloid content, individual alkaloid content, and nitrogen content. A *t*-test was conducted to analyze the relationship between herbivory level and total alkaloid and N concentrations. *Post hoc* comparisons for "region^ or "herbivory level^ were conducted using Tukey's HSD test. The association between alkaloids and intensity of *A. alstroemeriana* herbivory was tested by conducting a simple regression analysis between total alkaloid concentrations and number of leaf rolls and a multiple regression analysis with coniine, γ-coniceine, conhydrinone, and RT 12 as independent variables and leaf roll number as the dependent variable. Data were log transformed to fit normality when necessary.

RESULTS

Herbivory. A. alstroemeriana herbivory levels on *C. maculatum* were lower in Illinois compared with New York and Washington, ranging from absence of leaf rolls (level 0) to minor defoliation (level 2) (Figure 2). In New York, herbivory damage ranged from minor defoliation (level 2) to total defoliation (level 5). However, in New York, especially at the Etna site (ET), the sampling was conducted earlier in the season compared with the other regions, as shown by the number of early instars found, and thus the actual damage to plants may be higher over the course of the season than that estimated here. In Washington, the damage inflicted by herbivores was severe, ranging from major (level 4) to complete defoliation (level 5) (Figure 2), and plants in many stands were found to be totally desiccated as a result of the damage by *A. alstroemeriana*.

Plant Chemistry. We found a total of four alkaloids in *C. maculatum* foliage: coniine, γ-coniceine, conhydrinone, and an unknown alkaloid (RT 12) (Table 1). Not all compounds were present in every plant, and when present,

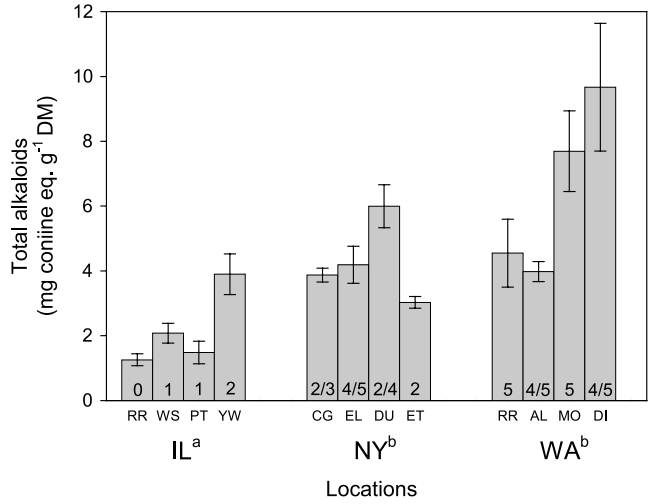


FIG. 2. Total alkaloid concentrations (mean ± SE) of *C. maculatum* plants by site in each region. In Illinois (IL): Railroad (RR), Windsor Rd (WS), Phillips Tract (PT), and Yard Waste (YW). In New York (NY): Coy Glenn (CG), Elm St (EL), Dump (DU), and Etna (ET). In Washington (WA): Railroad (RR), Albion (AL), Moscow (MO), and Ditch (DI). Averaged *Agonopterix alstroemeriana* herbivory levels for each site are indicated inside the graph bars (see a description of each level in Methods and Materials). Different letters indicate significant differences among regions at $P < 0.05$ by Tukey's *post hoc* comparisons test.

their concentrations were highly variable among individuals. Total alkaloid concentrations in plants expressed on a dry weight basis varied among the three regions (ANOVA, $P < 0.05$) and were lower in Illinois than in New York and Washington (Figure 2, Table 1). These lower levels reflected primarily lower concentrations of γ-coniceine, the major alkaloid in all three regions, constituting 80, 91, and 89% of the total alkaloids in Illinois, New York, and Washington, respectively. Plants in New York and Washington, although not different in total alkaloid content, differed in the concentrations of conhydrinone and RT 12, with lower values of both compounds in New York (Table 1). Coniine was present in about 16% of the plants growing in Illinois; however, in these plants, coniine reached such high concentrations that it constituted the second most abundant alkaloid after γ-coniceine. Coniine was not present either in New York or Washington (Table 1). Alkaloid concentrations expressed by unit N were significantly lower in Illinois and higher in Washington, partially because of differences in leaf N concentrations among regions (Figure 3). Total alkaloid concentrations were significantly higher in plants under increasing

TABLE 1. TOTAL ALKALOID CONCENTRATIONS (mg g^{-1} DM), INDIVIDUAL ALKALOID CONCENTRATIONS (mg g^{-1} DM), AND ALKALOID RELATIVE ABUNDANCE (%) IN *Conium maculatum* FROM ILLINOIS (IL), NEW YORK (NY), AND WASHINGTON (WA)

	Retention time (min)	IL	NY	WA
Total alkaloids		2.49[a]*	4.07[b]	6.48[b]
Coniine	5.7	[1.1][a] [(50.9%)]	0[b]	0[b]
γ-Coniceine	6.5	2.0[a] (80.8%)	3.96[b] (97.3%)	5.94[b] (91.7%)
Conhydrinone	10.0	0.22[a] (9.02%)	0.08[a] (1.96%)	0.37[b] (5.72%)
RT 12	11.9	0.07[a] (2.73%)	0.03[b] (0.68%)	0.16[c] (2.51%)

*Different letters indicate significant differences among regions at $P < 0.05$ by Tukey's *post hoc* comparisons test. Values of coniine shown in brackets indicate the average concentrations for the 16% of plants from the Illinois population that contain those alkaloids.

levels of *A. alstroemeriana* herbivory (Figure 4). No significant trends were found for herbivory levels relative to N concentrations (Figure 4).

Herbivory and Alkaloids. The number of leaf rolls in plants from Illinois, used as an estimate of *A. alstroemeriana* herbivory, was marginally negatively

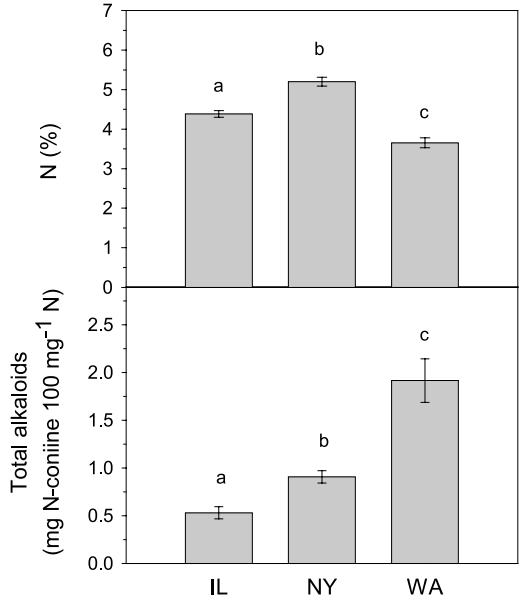
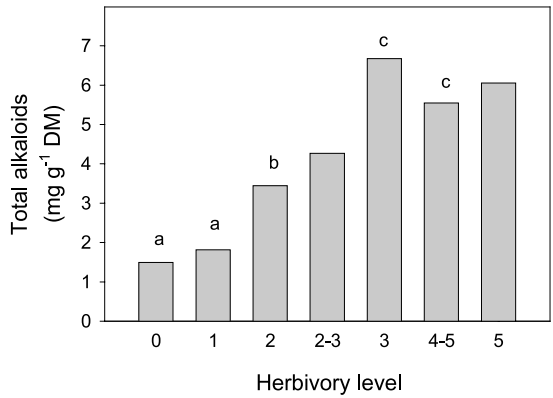


FIG. 3. Total N and total alkaloid concentrations (mean ± SE) expressed on N basis of *C. maculatum* growing in Illinois (IL), New York (NY), and Washington (WA). Different letters indicate significant differences at $P < 0.05$ by Tukey's *post hoc* comparisons.



correlated with total alkaloids (Figure 5a). In a multiple regression analysis, the number of leaf rolls was significantly correlated with γ-coniceine (Figure 5b), but no significant relationships were found for coniine, conhydrinone, or RT 12 (data not shown).

DISCUSSION

Poison hemlock has been established in Illinois for over 100 yr (Vasey, 1861; Patterson, 1876; Jones and Fuller, 1955). Despite the fact that the plant is extremely abundant locally, intermittent inspection over the last decade has consistently revealed few insect associates and little leaf damage by herbivores (personal observation). This same pattern has been documented in other parts

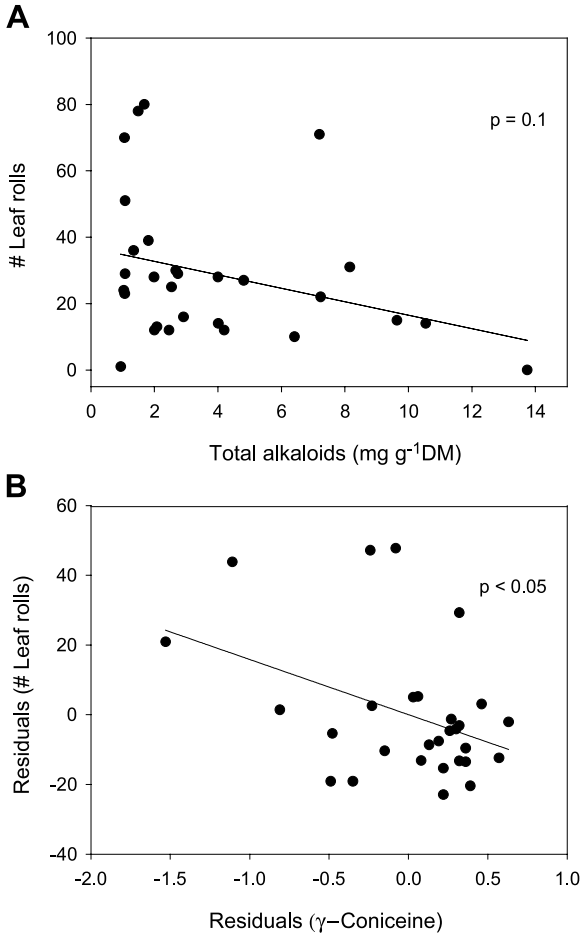


FIG. 5. (A) Simple regression between total alkaloid concentration and number of leaf rolls of 29 *C. maculatum* plants from Illinois. (B) Correlation between the residuals of γ-coniceine and the residuals of leaf roll number after performing multiple regression analyses with the independent variables coniine, γ-coniceine, conhydrinone, and RT 12.

of the United States V by Berenbaum (1981) in central New York and by Goeden and Ricker (1982) in southern California. Failure of insects to colonize this plant in large numbers over historical time may be attributable to its formidable array of chemical defenses. However, populations of poison hemlock in several localities in the United States are now experiencing unprecedented levels of herbivory because of colonization by and population

growth of *A. alstroemeriana*. It is of ecological and evolutionary interest to monitor changes in plant secondary compounds that may accompany this colonization. In this study, the chemistry of poison hemlock populations largely free from *A. alstroemeriana* (Illinois populations) was compared to populations in which *A. alstroemeriana* has become successfully established (New York and Washington populations) to determine under field conditions whether these herbivores, touted as potential biocontrol agents (http://www.bio-control.com, http://www.integratedweedcontrol.com), could reduce plant fitness and at the same time select for chemically based resistance factors (Berenbaum et al., 1986).

Changes in plant resource allocation between growth and chemical defenses driven by herbivore selective pressures have been discussed by the "optimal defense^ hypothesis (Zangerl and Bazzaz, 1992) and the derivative "evolution of increased competitive ability^ hypothesis, formulated in the context of invasion biology (Blossey and Nörtzold, 1995). According to these theoretical frameworks, when a plant species invades a new habitat where its native herbivores are absent, those genotypes with higher investments in growth and/or reproduction and reduced investments in defense are expected to have higher fitness and to increase their frequencies in the population. A logical corollary to this hypothesis is that defense investments should increase in the area of introduction commensurate with increases in herbivory, either by newly colonizing native species or by reassociation with introduced enemies from the area of indigeneity (as in the case of biological control). With the reassociation between the plant and the herbivore, such is the case of *C. maculatum* and *A. alstroemeriana*, the genotypes with increased levels of chemical defense should be favored by selection, particularly if, as is suggested in our study, high levels of alkaloid defense are deterrent to *A. alstroemeriana*. We found that C. maculatum plants from populations experiencing high levels of *A. alstroemeriana* herbivory (New York and Washington) had higher alkaloid concentrations in foliage than plants under lower levels of herbivory (Illinois). Because alkaloid concentrations in other species tend to increase with higher plant N availability (Gershenzon, 1984), concentrations in *C. maculatum* foliage were also analyzed to determine whether variation in the geographic pattern in *C. maculatum* alkaloid production could be related to differences in soils among regions. Nitrogen concentrations in the three populations varied significantly, with higher concentrations in New York and lower concentrations in Washington, but these differences were not consistent with the observed differences in alkaloid concentrations. Plants from New York and Washington invested two and four times more N to alkaloid synthesis, respectively, compared with plants from Illinois. In Washington populations, higher allocation of N to alkaloids was accompanied by lower total N concentration in foliage, which is suggestive of selection for higher constitutive or inducible alkaloid levels under more intense herbivory. Mechanistically, this pattern may

result from greater mortality on or avoidance of high levels of alkaloids by *A. alstroemeriana*; the negative relationship between the number of leaf rolls, and thus herbivory intensity, and the total alkaloid concentrations or γ-coniceine concentrations in Illinois plants is consistent with an increase in larval mortality with higher alkaloid concentrations.

Other environmental factors, such as water availability, could contribute to variation in the concentrations of alkaloids in *C. maculatum* across the United States. The different levels of alkaloid concentrations observed may reflect differential induced responses to herbivory (Castells et al., unpublished). Future studies, including a common garden experiment, will be necessary to determine definitively whether the geographic differences in alkaloids are genetically based and the result of a selection response to herbivory.

Although it was not deliberately introduced into the United States for biological control, *A. alstroemeriana* has demonstrated potential for systematic use as a biocontrol agent for poison hemlock. In the western United States, this insect has quickly become established naturally in infested locations and has established itself when it has been intentionally released (Piper, personal communication). Where it is established, it causes severe injury, including complete defoliation and destruction of inflorescences (Anonymous, 1995). However, the value of a biological control agent must be assessed not only by its ecological effect on population sizes, but also by its evolutionary impact on its target host plants. This study suggests that successful biological control agents may have the potential to alter the chemistry of their host plant, leading to increased allelochemical content, and potentially greater toxicity to livestock and humans who mistakenly ingest it.

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